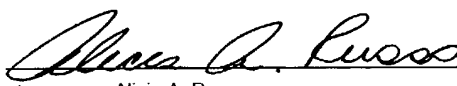
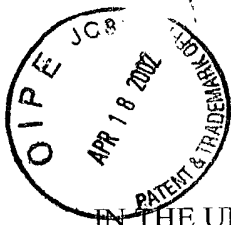


1.000.340.000 - 44.1.000
JCO3 Rec'd PCT/PTO 11 JAN 2002

BAKER BOTTS LLP TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35.U.S.C. 371		EXPRESS MAIL LABEL No. EF 377 398 644 US	DATE January 11, 2002
		ATTORNEY'S DOCKET NO. 34920-PCT-USA 072667.0179	
		U.S. APPLICATION NO. 10/030829	
INTERNATIONAL APPLICATION NO. PCT/FR00/02052	INTERNATIONAL FILING DATE July 13, 2000	PRIORITY DATE CLAIMED July 16, 1999	
TITLE OF INVENTION NOVEL SGS3 PLANT GENE AND USES THEREOF			
APPLICANT(S) FOR DO/EO/US Christophe BECLIN, Taline ELMAYAN, and Hervé VAUCHERET			
<p>Applicant herewith submits to the United States Designated /Elected Office (DO/EO/US) the following items and other information:</p> <ol style="list-style-type: none"> <input checked="" type="checkbox"/> This is a FIRST submission of items concerning a filing under 35 U.S.C. 371. <input type="checkbox"/> This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371. <input checked="" type="checkbox"/> This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(I). <input checked="" type="checkbox"/> A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date. <input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. 371(c)(2)) <ol style="list-style-type: none"> <input type="checkbox"/> is transmitted herewith (required only if not transmitted by the International Bureau). <input checked="" type="checkbox"/> has been transmitted by the International Bureau. <input type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US). <input type="checkbox"/> A translation of the International Application into English (35 U.S.C. 371(c)(2)). <input checked="" type="checkbox"/> A copy of the International Search Report (PCT/ISA/210) <ol style="list-style-type: none"> <input checked="" type="checkbox"/> are transmitted herewith (required only if not transmitted by the International Bureau). <input type="checkbox"/> have been transmitted by the International Bureau <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired. <input type="checkbox"/> have not been made and will not be made. <input type="checkbox"/> A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)). <input type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)). <input type="checkbox"/> A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)). <p>Items 11. to 16. below concern other document(s) or information included:</p> <ol style="list-style-type: none"> <input checked="" type="checkbox"/> A copy of the International Preliminary Examination Report (PCT/IPEA/409) <input type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included. <input type="checkbox"/> A FIRST preliminary amendment. <input type="checkbox"/> A SECOND or SUBSEQUENT preliminary amendment. <input type="checkbox"/> A substitute specification. <input type="checkbox"/> A change of power of attorney and/or address letter. <input type="checkbox"/> Other items or information: <ol style="list-style-type: none"> <input type="checkbox"/> a copy of the International Search Report (PCT/ISA/210) <input type="checkbox"/> a copy of the International Preliminary Examination Report (PCT/IPEA/409) 			

531 Rec'd PCT/ITC 11 JAN 2002

INTERNATIONAL APPLICATION NO. 10/030829 INTERNATIONAL FILING DATE PCT/FR00/02052 July 13, 2000				PRIORITY DATE CLAIMED July 16, 1999	
17. [] The following fees are submitted: Basic National Fee (37 CFR 1.492(a)(1)-(5)): Neither international preliminary examination fee (37 CFR 1.482) Nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO (1.492(a)(3)) \$1,040 International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO (1.492(a)(5)) \$890.00 International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO (1.492(a)(2)) \$740.00 International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) (1.492(a)(1)) \$710.00 International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) \$100.00 <div style="text-align: right;">ENTER APPROPRIATE BASIC FEE AMOUNT = \$ 890</div>				CALCULATIONS <small>PTOUSE ONLY</small>	
Surcharge of \$130.00 for furnishing the oath or declaration later than [] 20 <input checked="" type="checkbox"/> 30 months from the earliest claimed priority date (37 C.F.R. 1.492(e)).				\$ 130	
Claims	Number Filed	Number Extra	Rate		
Total Claims	61 -20=	41	X \$ 18.00	\$ 738	
Independent Claims	4 -3=	1	X \$ 84.00	\$ 84	
Multiple dependent claim(s) (if applicable)			+ \$280.00	\$ 280	
TOTAL OF ABOVE CALCULATIONS				= \$ 2,122	
Reduction by 1/2 for filing by small entity, if applicable.				\$	
SUBTOTAL				= \$ 2,122	
Processing fee of \$130.00 for furnishing the English translation later than [] 20 <input checked="" type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				\$ 130	
TOTAL NATIONAL FEE				= \$ 2,252	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property +				\$	
TOTAL FEES ENCLOSED				= \$ 2,252	
				Amt. refunded \$	
				charged \$	
a. [] A check in the amount of \$ 2,252.00 to cover the above fees is enclosed. b. [] Please charge our Deposit Account No. <u>02-4377</u> in amount of \$_____ to cover the above fees. A copy of this sheet is enclosed. c. <input checked="" type="checkbox"/> The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. <u>02-4377</u> . A copy of this sheet is enclosed.					
NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.					
SEND ALL CORRESPONDENCE TO: Alicia A. Russo BAKER BOTTS L.L.P. 30 Rockefeller Plaza New York, New York 10112-4498			 Attorney: Alicia A. Russo		
			PTO Reg: 46,192		
			January 11, 2002		
			Date		



10 Resubmitted 18 APR 2002

A34920-PCT-USA 072667.0179

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Christophe Beclin et al.
Serial No. : 10/030,829 Examiner : TBA
Filed : January 11, 2002 Group Art Unit : TBA
For : NOVEL SGS3 PLANT GENE AND USES THEREOF

PRELIMINARY AMENDMENT

I hereby certify that this paper is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231

April 11, 2002

Date of Deposit

Alicia A. Russo

Name

46,192

PTO Reg. No.



Signature

April 11, 2002

Date of Signature

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Applicants respectfully request entry of the following amendments prior to examination on the merits pursuant to 37 C.F.R. §1.115(b)(2)(iii). Applicants enclose herewith an English translation of the international application pursuant to 37 C.F.R. §1.495(c)(1). Applicants also enclose a Substitute Sequence Listing in paper and electronic form, a Combined Declaration and Power of Attorney, and an Assignment and enclose the fees required pursuant to

37 C.F.R. §1.121(h). The fees required pursuant to 37 C.F.R. §1.16(e) and 37 C.F.R. §1.492(f) have been submitted previously.

IN THE SPECIFICATION

Please **delete** the Sequence Listing presently of record and substitute, therefor, the attached Substitute Sequence Listing.

Please **amend** the paragraph beginning on page 4, line 14 (of the English translation) and ending on page 4, line 18 with the following rewritten paragraph:

Description of the Sequence Listing

SEQ ID NO:1: *SGS3* gene of *Arabidopsis thaliana*.
SEQ ID NO:2: cDNA of the *SGS3* gene of *Arabidopsis thaliana*.
SEQ ID NO:3: *SGS3* polypeptide of *Arabidopsis thaliana*.
SEQ ID NO:4: Primer p356AD'.
SEQ ID NO:5: Primer p356Y'.

Please **amend** the paragraph beginning on page 47, line 1 (of the English translation) and ending on page 47, line 22 with the following rewritten paragraph:

The DNA sequence which was inserted at the BamHI site of the pBin+ plasmid and which had led to the isolation of the bacterial strain 356 was determined. Subclones of the 356 clone were produced in the pBin+ vector and the same *sgs3-2 2a3* line was transformed with these subclones in order to determine those

IN THE CLAIMS

Please **add** the following new claims:

- 3

24. (NEW) The isolated nucleic acid of claim 23 wherein said nucleotide sequence is at least 90% homologous to the reference sequence.

26. (NEW) The isolated nucleic acid of claim 25 wherein said nucleotide sequence is at least 98% homologous to the reference sequence.

28. (NEW) The isolated nucleic acid of claim **23** wherein said reference sequence is nucleotides 1-695 of SEQ ID NO:1.

NY02:380375.1

30. (NEW) The isolated nucleic acid of claim 28 wherein said nucleic acid has promoter activity in a plant cell or a plant.
31. (NEW) An isolated nucleic acid comprising a nucleotide sequence having nucleotides 1-695 of SEQ ID NO:1.
32. (NEW) An isolated nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NO:1 and SEQ ID NO:2.
33. (NEW) The isolated nucleic acid of claim 32 wherein said nucleotide sequence is SEQ ID NO:1.
34. (NEW) The isolated nucleic acid of claim 32 wherein said nucleotide sequence is SEQ ID NO:2.
35. (NEW) The isolated nucleic acid of claim 29 wherein said nucleic acid restores an sgs3 mutant of *Arabidopsis thaliana*.
36. (NEW) An isolated polypeptide comprising an amino acid sequence having at least 80% homology to SEQ ID NO:3.
37. (NEW) The isolated polypeptide of claim 36 wherein said amino acid sequence is at least 90% homologous to SEQ ID NO:3.
38. (NEW) The isolated polypeptide of claim 37 wherein said amino acid sequence is at least 95% homologous to SEQ ID NO:3.

- NY02:380375.1

45. (NEW) An expression cassette comprising:

a plant promoter;

a nucleic acid comprising a nucleotide sequence that is
at least 80% homologous to the complement of a
nucleotide sequence selected from the group consisting
of SEQ ID NO:1 and SEQ ID NO:2; and

a plant terminator,

wherein said plant promoter is operably linked to said nucleic
acid, and wherein said terminator is operably linked to said
nucleic acid.

46. (NEW) An expression cassette comprising:

a plant promoter having a nucleotide sequence that is at
least 80% homologous to nucleotides 1-695 of SEQ ID
NO:1,

a nucleic acid encoding a heterologous polypeptide, and
a plant terminator,

wherein said plant promoter is operably linked to said nucleic
acid, and wherein said terminator is operably linked to said
nucleic acid.

47. (NEW) An expression vector or transformation vector
comprising a nucleic acid of claim 23, 28, or 29 or an
expression cassette of claim 44, 45, or 46.

48. (NEW) A process for transforming a host organism comprising contacting the host organism with either a nucleic acid of claim 23, 28, or 29 or an expression cassette of claim 44, 45, or 46.

49. (NEW) A process for expressing a heterologous gene in a host organism comprising contacting a host organism, comprising a heterologous gene, with an expression cassette comprising:

a plant promoter;

a nucleic acid comprising a nucleotide sequence that is at least 80% homologous to the complement of a nucleotide sequence selected from the group consisting of SEQ ID NO:1 and SEQ ID NO:2; and a plant terminator.

50. (NEW) A process for expressing a heterologous gene in a host organism comprising contacting a host organism which comprises a heterologous gene, with a polypeptide comprising an amino acid sequence that is at least 80% homologous to SEQ ID NO:3.

51. (NEW) A transformed host organism comprising at least one nucleic acid of claim 23, 28, or 29 or an expression cassette of claim 44, 45, or 46.

52. (NEW) An isolated nucleic acid that selectively hybridizes to a nucleic acid having a nucleotide sequence selected from the group consisting of nucleotides 1-695 of SEQ ID NO:1, SEQ ID NO:1, SEQ ID NO:2, and the complements thereof.

REMARKS

Applicants respectfully request entry of the following amendments prior to examination on the merits pursuant to 37 C.F.R. §1.115(b)(2)(iii). Applicants enclose herewith an English translation of the international application pursuant to 37 C.F.R. §1.495(c)(1). Applicants also enclose a Substitute Sequence Listing in paper and electronic form, a Combined Declaration and Power of Attorney, and an Assignment together with the fees required pursuant to 37 C.F.R. §1.21(h). The fees required pursuant to 37 C.F.R. §1.16(e) and 37 C.F.R. §1.492(f) have been submitted previously.

Claims 1-22 are pending. Claims 1-22 have been cancelled and new claims 23-52 have been added. Applicants assert that the new claims are fully supported by the application as originally filed and, therefore, do not constitute new matter. Specifically, claims 23-52 are supported by, *inter alia*, original claims 1-22.

Rewritten paragraphs appear in the preceding "IN THE SPECIFICATION" section. Attached hereto is a marked-up version of the changes made to the specification paragraphs by the instant amendment captioned "VERSION WITH MARKINGS TO SHOW CHANGES MADE" and is included pursuant to 37 C.F.R. §1.121(c)(ii). Should any discrepancies be discovered, the version presented in the preceding "IN THE SPECIFICATION" section shall take precedence.


A sequence listing in computer readable form has not previously been filed in this application. Nevertheless, both the electronic and paper sequence listing attached hereto are identified as "Substitute Sequence Listing."

I hereby state that the content of the paper and computer readable copies of the Substitute Sequence Listing submitted in accordance with 37 C.F.R. §1.821(c) and (e), are the same. I hereby state that the content of the paper and computer readable copies of the Substitute Sequence Listing, submitted in accordance with 37 C.F.R. §1.821(g), herein does not include new matter.

The Commissioner is hereby authorized to charge any fees due with this submission not otherwise enclosed herewith to Deposit Account No. 02-4377. Please credit any overpayment of fees associated with this filing to the above-identified deposit account. A duplicate of this page is enclosed.

Respectfully submitted,

April 11, 2002


Louis S. Sorell
PTO Reg. No. 32,439

Alicia A. Russo
PTO Reg. No. 46,192
Attorneys for Applicant

BAKER BOTTS, L.L.P.
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Enclosures

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

This marked-up version was prepared with DeltaView software (v2.5.163). In this section, added text is marked with double underlining. *e.g.* added text, and deleted text is marked by a single strikethrough, *e.g.* ~~deleted text~~.

IN THE SPECIFICATION

The paragraph beginning on page 4, line 14 (of the English translation) and ending on page 4, line 18 has been **amended** as follows:

Description of the Sequence Listing

SEQ ID No. ~~NO~~:1: *SGS3* gene of *Arabidopsis thaliana*.

SEQ ID No. ~~NO~~:2: cDNA of the *SGS3* gene of *Arabidopsis thaliana*.

SEQ ID No. ~~NO~~:3: *SGS3* polypeptide of *Arabidopsis thaliana*.

SEQ ID NO:4: Primer p356AD'.

SEQ ID NO:5: Primer p356Y'.

The paragraph beginning on page 47, line 1 (of the English translation) and ending on page 47, line 22 has been **amended** as follows:

The DNA sequence which was inserted at the BamHI site of the pBin+ plasmid and which had led to the isolation of the bacterial strain 356 was determined. Subclones of the 356 clone were produced in the pBin+ vector and the same *sgs3-2 2a3* line was transformed with these subclones in order to determine those

capable of restoring the function of the *SGS3* gene. The smallest subclone capable of restoring this function constitutes the *SGS3* gene such as it is described in this patentdisclosure. It was possible to predict the ORF of *SGS3* by computer analysis. The sequence of the cDNA containing the ORF of the *SGS3* gene, and therefore the position of the promoter, terminator and intronic sequences of *SGS3*, were verified after having isolated and cloned this sequence. In order to isolate, we first performed a reverse transcription reaction using *Arabidopsis thaliana* total RNA. We then performed a PCR reaction on this pool of cDNA using the pair of primers p356AD' (AAAATGAGTTCTAGGGCTGGTCC; SEQ ID NO:4) and p356Y' (GTCTCAATCATCTTCATTGTGAAGGCC; SEQ ID NO:5). These primers are located at the 2 ends of the ORF of *SGS3*. This PCR product was cloned and sequenced.

Novel SGS3 plant gene and use thereof

The present invention relates to a novel SGS3
plant gene and use thereof for preparing genetically
5 modified plants.

Methods are known, from the state of the art,
which make it possible to integrate heterologous genes
into the genome of plants of various species. For the
processes for transforming plant cells and for
10 regenerating plants, mention will in particular be made
of the following patents and patent applications:
US 4,459,355, US 4,536,475, US 5,464,763, US 5,177,010,
US 5,187,073, EP 267 159, EP 604 662, EP 672 752,
US 4,945,050, US 5,036,006, US 5,100,792, US 5,371,014,
15 US 5,478,744, US 5,179,022, US 5,565,346, US 5,484,956,
US 5,508,468, US 5,538,877, US 5,554,798, US 5,489,520,
US 5,510,318, US 5,204,253, US 5,405,765, EP 442 174,
EP 486 233, EP 486 234, EP 539 563, EP 674 725,
WO 91/02071 and WO 95/06128.

20 The level of expression of the heterologous
gene will depend on various factors, including the
locus of integration of the heterologous gene into the
genome of the transformed plant and "silencing"
phenomena. It is, in fact, known from the state of the
25 art that the expression of a heterologous gene in a
plant may be totally or partially inhibited in the
descendents of the regenerated transformed plants, even

though said gene is expressed correctly in the regenerated plant directly derived from the transformed cell. The heterologous genes introduced may sometimes undergo epigenetic inactivation (inactivation
5 accompanied by no sequence modification). When the genes exhibit homology with genes of the host organism, the inactivation may also affect the expression of these host genes and engender effects which are deleterious for the organism (co-inactivation). Two
10 distinct inactivation mechanisms have been demonstrated in higher plants, resulting either in blocking of transcription (transcriptional inactivation) or in RNA degradation (post-transcriptional inactivation).

These inactivation phenomena, accidentally
15 revealed by transgenesis, certainly reflect fundamental processes for the epigenetic control of gene expression, and their study therefore constitutes an original means of access to understanding the regulatory mechanisms used during plant development.
20 The demonstration of these phenomena raises, moreover, many questions regarding the use of transgenic plants both for variety improvement programs and for molecular physiology studies.

Thus, monolocus homozygous plants obtained
25 with a gene encoding the GUS protein under the control of the promoter CamV 35S (35S-UidA) have exhibited inactivation of the transgene, regardless of the number of copies of the transgene inserted at the locus. The

A novel plant gene, named *SGS3*, has now been isolated, which is involved in post-transcriptional inactivation phenomena in transgenic plants, and in the resistance of plants to viral infections. Inhibition of this gene leads to inhibition of the post-transcriptional inactivation phenomena, in particular in transgenic plants comprising a heterologous gene encoding a particular peptide or protein, allowing a particularly high level of expression of said peptide or of said protein. A subject of the invention is also the overexpression of the *SGS3* gene, for preparing plants which are more resistant to viral infections.

Description of the sequence listing

- SEQ ID No. 1: *SGS3* gene of *Arabidopsis thaliana*
SEQ ID No. 2: cDNA of the *SGS3* gene of *Arabidopsis thaliana*
SEQ ID No. 3: *SGS3* polypeptide of *Arabidopsis thaliana*

Description of the invention

SGS3 polynucleotides

The present invention relates to *SGS3* polynucleotides, in particular polynucleotides comprising an *SGS3* plant gene. Preferentially, the polynucleotides of the present invention comprise the coding sequence of an *SGS3* plant gene. The *SGS3* gene may be isolated from dicotyledon plants, such as *Arabidopsis*, tobacco, rapeseed, sunflower, soybean,

conventional molecular biology techniques as described by Sambrook et al. (Molecular Cloning: A Laboratory Manual, 1989), or by chemical synthesis.

The invention comprises polynucleotides of the genomic sequence of the *SGS3* gene. This genomic sequence comprises 5 exons (positions 696-1658, 1732-2023, 2135-2379, 2482-2648 and 2739-2949 of SEQ ID No. 1), 4 introns (positions 1659-1731, 2024-2134, 2380-2481 and 2649-2738 of SEQ ID No. 1), and 5' and 3' regulatory sequences. In a preferred embodiment of the invention, the polynucleotides of the genomic sequence of *SGS3* comprise a polynucleotide chosen from the following polynucleotides:

- a) the polynucleotide of SEQ ID No. 1,
- b) a polynucleotide comprising at least one exon of SEQ ID No. 1,
- c) a polynucleotide comprising a combination of exons of SEQ ID No. 1.

The present invention also relates to a polynucleotide comprising a 5' or 3' regulatory sequence of the *SGS3* gene. In a first embodiment, the invention relates to a 5' regulatory polynucleotide comprising the polynucleotide the sequence of which is between position 1 and position 695 of SEQ ID No. 1. In a second embodiment, the invention relates to a 3' regulatory polynucleotide comprising the polynucleotide the sequence of which is between position 2950 and position 3275 of SEQ ID No. 1.

b) a polynucleotide capable of selectively hybridizing to a polynucleotide the sequence of which is between position 1 and position 695 of SEQ ID No. 1. Preferably, these polynucleotides have promoter activity in plant cells and plants.

The invention also relates to a terminator sequence of the *SGS3* gene of *Arabidopsis thaliana*. Preferably, the *SGS3* gene terminator sequence comprises a polynucleotide the sequence of which is between position 2950 and position 3275 of SEQ ID No. 1. In another embodiment of the invention, the *SGS3* gene terminator sequence comprises a biologically active fragment of a polynucleotide the sequence of which is between position 2950 and position 3275 of SEQ ID No. 1.

The invention also relates to polynucleotides of the *SGS3* cDNA. Preferably, the polynucleotides of the coding sequence of an *SGS3* plant gene comprise polynucleotides of SEQ ID No. 2.

The invention also extends to the polynucleotides comprising a polynucleotide chosen from the following polynucleotides:

- a) a polynucleotide homologous to a polynucleotide according to SEQ ID No. 1 or SEQ ID No. 2;
- b) a polynucleotide capable of selectively hybridizing to a polynucleotide according to SEQ ID No. 1 or SEQ ID No. 2.

Preferably, the polynucleotides homologous to a reference polynucleotide, or selectively hybridizing to a reference polynucleotide, conserve the function of the reference sequence. The polynucleotides of the present invention preferably encode a polypeptide essential for post-transcriptional inactivation in plants. Preferentially, the polynucleotides of the present invention restore an *sgs3* mutant of *Arabidopsis thaliana*. These mutants, and the method for producing them, are described in Elmayan et al. (Plant Cell, 10:1747-1757, 1998). Other methods which make it possible to construct *Arabidopsis thaliana* mutants in which the *SGS3* gene is inactivated are well known to those skilled in the art. The methods for producing *Arabidopsis thaliana* mutants are widely described in the literature.

According to the invention, the term "homolog" is intended to mean a polynucleotide having one or more sequence modifications compared to the reference sequence. These modifications may be deletions, additions or substitutions of one or more nucleotides of the reference sequence. Advantageously, the percentage homology will be at least 70%, 75%, 80%, 85%, 90%, 95%, and preferably at least 98%, and more preferentially at least 99%, compared to the reference sequence. The methods for measuring and identifying homologies between nucleic acid sequences are well known to those skilled in the art. Use may be made, for

example, of the PILEUP or BLAST programs (in particular Altschul et al., J. Mol. Evol., 36:290-300, 1993; Altschul et al., J. Mol. Biol., 215:403-10, 1990). The invention therefore relates to polynucleotides

5 comprising polynucleotides exhibiting at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, and preferably at least 98%, and more preferentially at least 99%, homology with the SGS3 polynucleotides, the polynucleotides of SEQ ID No. 1 or the polynucleotides of SEQ ID No. 2.

10 Preferably, the invention relates to a polynucleotide comprising a polynucleotide of at least 50, 100, 200, 300, 400, 500, 1000 nucleotides, exhibiting at least 70%, 75%, 80%, 85%, 90%, 95%, 98% and preferably at least 98%, and more preferentially at least 99%,

15 homology with the SGS3 polynucleotides, the polynucleotides of SEQ ID No. 1 or the polynucleotides of SEQ ID No. 2. Preferably, these homologs conserve the function of the reference sequence.

According to the invention, the expression

20 "sequence capable of selectively hybridizing" is intended to mean the sequences which hybridize with the reference sequence at a level significantly greater than the background noise. The level of the signal generated by the interaction between the sequence

25 capable of selectively hybridizing and the reference sequences is generally 10 times, preferably 100 times, more intense than that of the interaction of the other DNA sequences generating the background noise. The

stringent hybridization conditions which allow selective hybridization are well known to those skilled in the art. In general the temperature for hybridization and for washing is at least 5°C lower than the T_m of the reference sequence at a given pH and for a given ionic strength. Typically, the hybridization temperature is at least 30°C for a polynucleotide of 15 to 50 nucleotides, and at least 60°C for a polynucleotide of more than 50 nucleotides.

By way of example, the hybridization is carried out in the following buffer: 6X SSC, 50 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.02% PVP, 0.02% Ficoll, 0.02% BSA and 500 µg/ml denatured salmon sperm DNA. The washes are, for example, carried out successively at low stringency in a 2X SSC buffer containing 0.1% SDS, at medium stringency in a 0.5X SSC buffer containing 0.1% SDS and at high stringency in a 0.1X SSC buffer containing 0.1% SDS. The hybridization may, of course, be carried out according to other normal methods well known to those skilled in the art (in particular Sambrook et al., Molecular Cloning: A Laboratory Manual, 1989). The invention therefore relates to polynucleotides comprising a polynucleotide capable of selectively hybridizing with the polynucleotide of SEQ ID No. 1 or the polynucleotide of SEQ ID No. 2. Preferably, the invention relates to a polynucleotide comprising a polynucleotide of at least 50, 100, 200, 300, 400, 500, 1000 nucleotides, capable of selectively hybridizing

with the polynucleotide of SEQ ID No. 1 or the polynucleotide of SEQ ID No. 2. Preferentially, these polynucleotides selectively hybridizing to a reference polynucleotide conserve the function of the reference sequence.

A subject of the present invention is also antisense polynucleotides which allow inhibition of the expression of an *SGS3* plant gene. The antisense polynucleotides hybridize specifically to the mRNA of an *SGS3* plant gene, thus interfering with the expression of this gene. The techniques for inhibiting the expression of a protein with an antisense polynucleotide are well known to those skilled in the art and widely described in the literature, in particular by Judelson et al. (*Gene*, 133:63-69, 1993) and also by Prokish et al. (*Mol. Gen. Genet.* 256:104-114, 1997).

The antisense polynucleotides of the present invention hybridize to the mRNA of an *SGS3* plant gene over its entire length, or only to a part of the mRNA of an *SGS3* plant gene. The antisense polynucleotides of the present invention may be completely complementary to the mRNA of an *SGS3* plant gene, or sufficiently homologous to allow pairing and inhibition of the expression of an *SGS3* plant gene.

A subject of the present invention is therefore also polynucleotides comprising an antisense polynucleotide of an *SGS3* plant gene, and

preferentially an antisense polynucleotide of the coding sequence of the *SGS3* gene of SEQ ID No. 2. Preferentially, the antisense polynucleotides of the present invention are derived from a polynucleotide of

5 SEQ ID No. 2. According to a first embodiment, the antisense polynucleotides of the present invention comprise the polynucleotide of SEQ ID No. 2. According to a second embodiment, the antisense polynucleotides of the present invention comprise a fragment of at

10 least 100 nucleotides, preferably of at least 500 nucleotides, and preferentially of at least 1000 nucleotides, of SEQ ID No. 2. According to a third embodiment, the antisense polynucleotides of the present invention comprise a polynucleotide exhibiting

15 at least 85%, 90%, 95%, and preferably at least 98%, and more preferentially at least 99%, homology with a polynucleotide of SEQ ID No. 2. According to another embodiment, the antisense polynucleotides of the present invention comprise a polynucleotide exhibiting

20 at least 85%, 90%, 95%, and preferably at least 98%, and more preferentially at least 99%, homology with a fragment of at least 100 nucleotides, preferably of at least 500 nucleotides, and preferentially of at least 1000 nucleotides, of SEQ ID No. 2.

25 Preferably, the antisense polynucleotides of the present invention specifically inhibit the expression of an *SGS3* gene in plants.

According to a preferred embodiment, the antisense polynucleotides of the present invention are expressed in plant cells or plants using an expression cassette.

5 The present invention relates to the use of a polynucleotide, or of a fragment of a polynucleotide, of SEQ ID No. 1 and of SEQ ID No. 2 according to the invention, for identifying the *SGS3* gene in other plants. The cloning is carried out, for example, by
10 screening cDNA libraries or genomic DNA libraries with a polynucleotide, or a fragment of a polynucleotide, of SEQ ID No. 1 and of SEQ ID No. 2. These libraries may also be screened by PCR using specific or degenerate oligonucleotides derived from SEQ ID No. 1 or from SEQ
15 ID No. 2. The techniques for constructing and screening these libraries are well known to those skilled in the art (see in particular Sambrook et al., Molecular Cloning: A Laboratory Manual, 1989). *SGS3* plant genes may also be identified in the databases by nucleotide
20 or protein BLAST using SEQ ID Nos. 1-3.

 Preferably, it is verified that the cloned genes carry out the same function as the *SGS3* gene of *Arabidopsis thaliana*, by introducing the genes identified into *SGS3* mutants and by testing for
25 restoration of the post-transcriptional inactivation (see below).

A subject of the invention is also polynucleotides comprising a polynucleotide encoding a polypeptide according to the invention.

5 SGS3 polypeptides

The present invention also relates to SGS3 polypeptides. The term "SGS3 polypeptides" denotes all of the polypeptides of the present invention, and also the polypeptides for which the polynucleotides of the present invention code. The term "SGS3 polypeptides" also denotes fusion proteins, recombinant proteins or chimeric proteins comprising these polypeptides. In the present description, the term "polypeptide" also denotes proteins and peptides, and also modified polypeptides.

The polypeptides of the invention are isolated or purified from their natural environment. The polypeptides may be prepared by means of various processes. These processes are, in particular, purification from natural sources such as cells naturally expressing these polypeptides, production of recombinant polypeptides by suitable host cells and subsequent purification thereof, production by chemical synthesis or, finally, a combination of these various approaches. The various production processes are well known to those skilled in the art. Thus, the SGS3 polypeptides of the present invention may be isolated from plants expressing SGS3 polypeptides. Preferably,

may have a deletion, an addition or a substitution of at least one amino acid. A subject of the invention is a polypeptide exhibiting at least 75%, 80%, 85%, 90%, 95%, 98%, and preferentially 99%, of amino acids
 5 identical to a polypeptide of SEQ ID No. 3. Preferably, these homologous polypeptides conserve the same biological activity. Preferentially, this activity relates to post-transcriptional inactivation in plants. Preferably, the polypeptides of the present invention
 10 restore an sgs3 mutant of *Arabidopsis thaliana*.

Expression cassettes

The *SGS3* gene may be expressed or overexpressed in various host organisms, such as
 15 plants. The present invention relates in particular to the overexpression of the *SGS3* gene in plants or plant cells in order to improve their resistance to viruses. The *SGS3* gene may be expressed in a host organism, under the control of the *SGS3* promoter of the present
 20 invention or under the control of a heterologous promoter, and preferably under the control of a promoter which is functional in plants. According to one embodiment of the invention, a polynucleotide encoding an *SGS3* polypeptide is inserted into an
 25 expression cassette using cloning techniques well known to those skilled in the art. This expression cassette comprises the elements required for the transcription and translation of the sequences encoding the *SGS3*

- polypeptide. Advantageously, this expression cassette comprises both elements for making a host cell produce an SGS3 polypeptide and elements required for regulating this expression. In a first embodiment, the
- 5 expression cassettes according to the invention comprise, in the direction of transcription, a promoter which is functional in a host organism, an SGS3 plant gene, or the coding sequence of an SGS3 plant gene, and a sequence which is a terminator in said host organism.
- 10 In another embodiment, the expression cassettes according to the invention comprise, in the direction of transcription, a promoter which is functional in a host organism, a polynucleotide encoding an SGS3 polypeptide and a sequence which is a terminator in
- 15 said host organism. Preferentially, the expression cassette comprises, in the direction of transcription, a promoter which is functional in a host organism, a polynucleotide chosen from the following polynucleotides:
- 20 a) a polynucleotide encoding an SGS3 polypeptide of SEQ ID No. 3, encoding a homolog or encoding a fragment of a polypeptide of SEQ ID No. 3;
- b) a polynucleotide of SEQ ID No. 1;
- 25 c) a polynucleotide of SEQ ID No. 2;
- d) a polynucleotide homologous to a polynucleotide as defined in b) or c);

- e) a polynucleotide capable of hybridizing specifically to a polynucleotide as defined in b) or c);
 - f) a polynucleotide comprising a fragment of a polynucleotide as defined in b), c), d) and e),
- and a sequence which is a terminator in said host organism.

In another embodiment, the expression cassettes of the present invention allow the expression of an antisense polynucleotide, for inhibiting the expression of the *SGS3* gene in a plant. For expressing an antisense polynucleotide in a plant, the expression cassettes according to the invention comprise, in the direction of transcription, a promoter which is functional in a host organism, an antisense polynucleotide of the coding sequence of an *SGS3* plant gene and a terminator sequence which is functional in said host organism. Preferably, the expression cassettes according to the invention comprise, in the direction of transcription, a promoter which is functional in a host organism, an antisense polynucleotide of the coding sequence of the *SGS3* gene of SEQ ID No. 2 and a terminator sequence which is functional in said host organism. Preferentially, the antisense polynucleotides of the present invention are expressed under the control of an inducible promoter.

In a preferred embodiment, the subject of the invention is an expression cassette comprising, in the direction of transcription:

- a) a promoter which is functional in a host organism;
- 5 and
- b) an SGS3 polynucleotide according to the invention in the antisense orientation; and
- c) a sequence which is a terminator in said host organism.

10 The SGS3 promoter may be used to express a heterologous gene in a host organism, and in particular in plant cells or in plants. A subject of the invention is therefore also expression cassettes comprising the promoter of an SGS3 plant gene, functionally combined

15 with a sequence encoding a heterologous protein, allowing the expression of said protein in plant cells or plants. In one embodiment, the expression cassette according to invention comprises, in the direction of transcription, the SGS3 promoter of *Arabidopsis*

20 *thaliana*, the coding sequence for the heterologous protein and a terminator sequence which is functional in plant cells and plants. Preferably, the expression cassette according to the invention comprises, in the direction of transcription, a polynucleotide the

25 sequence of which is between position 1 and position 695 of SEQ ID No. 1, or a biologically active fragment of the polynucleotide the sequence of which is between position 1 and position 695 of SEQ ID No. 1, the

sequence encoding a heterologous polypeptide and a terminator sequence which is functional in plant cells and plants.

The expression cassettes according to the present invention may also include any other sequence required for the expression of the gene of interest, such as, for example regulatory elements or signal sequences allowing the polypeptide of interest to be addressed.

The techniques for constructing these expression cassettes are widely described in the literature (see in particular Sambrook et al., Molecular Cloning: A Laboratory Manual, 1989).

A subject of the present invention is also a polynucleotide comprising an expression cassette according to the invention, and in particular a vector comprising an expression cassette according to the invention.

Advantageously, the expression cassettes according to the present invention are inserted into a vector for replicating them or for transforming a host organism.

Certain elements of the expression cassettes according to the invention are illustrated below in a nonlimiting manner.

Promoters

Any type of promoter sequence may be used in the expression cassettes according to the invention.

regions or tissues of plants, and more particularly promoters specific for seeds (Datla et al., Biotechnology Ann. Rev. 3:269-296, 1997). Use may also be made of an inducible promoter advantageously chosen

5 from the phenylalanine ammonia lyase (PAL), HMG-CoA reductase (HMG), chitinase, glucanase, proteinase inhibitor (PI) or PR1 family gene promoters, the nopaline synthase (nos) promoter, the vspB gene promoter (US 5,670,349), the HMG2 promoter

10 (US 5 670 349), the apple beta-galactosidase (ABG1) promoter or the apple aminocyclopropane carboxylate syntase (ACC synthase) promoter (WO 98/45445).

Use may also be made of the *SGS3* gene promoter of *Arabidopsis thaliana*.

15 Regulatory sequences for expression

In the expression cassettes of the present invention, use may be made of any regulatory sequence which makes it possible to increase the level of expression of the coding sequence inserted into said

20 expression cassette. According to the invention, use may in particular be made, in combination with the regulatory promoter sequence, of other regulatory sequences, which are located between the promoter and the coding sequence, such as transcription activators

25 ("enhancer"). Among the virus-derived leader sequences, mention will be made, for example, of the tobacco mosaic virus (TMV) activator described in application WO 87/07644, or the tobacco etch virus (TEV) activator.

Various plant intron-derived sequences may also be used to increase the level of expression of the gene of interest, in particular in monocotyledon plants. Mention will be made, for example, of intron I of the maize gene, AdhI (Callis et al., Genes Develop., 1:1183-1200, 1987).

Terminator sequences

A large variety of terminator sequences can be used in the expression cassettes according to the invention. These sequences allow transcription termination and polyadenylation of the mRNA. Any terminator sequence which is functional in the host organism selected may be used. For expression in plants, use may in particular be made of the *nos* terminator of *Agrobacterium tumefaciens*, or terminator sequences of plant origin, such as for example the histone terminator (see EP 0 633 317), the CaMV 35 S terminator and the *tml* terminator. These terminator sequences can be used in monocotyledon and dicotyledon plants.

The terminator sequence of the *SGS3* gene of *Arabidopsis thaliana* is another example of a terminator sequence which can be used in the expression cassettes according to the invention.

25 Heterologous genes

Any gene of interest may be expressed in a host organism under the control of an *SGS3* promoter. Preferably, the *SGS3* promoter is used for expressing a

heterologous gene in plant cells or in a plant. The genes of interest which may be expressed in plants under the control of an *SGS3* promoter are more widely illustrated below.

5

Vectors

The present invention also relates to transformation vectors or expression vectors comprising at least one *SGS3* polynucleotide or one expression
10 cassette according to the present invention. The vectors of the present invention are in particular used to transform a host organism and to express an *SGS3* polypeptide or an *SGS3* polynucleotide, in said host organism. The host organism is, for example, a
15 bacterium, a yeast, a fungus, a plant cell or a plant. This vector may in particular consist of a plasmid, a cosmid, a bacteriophage or a virus, into which an *SGS3* polynucleotide or an expression cassette according to the invention is inserted. In general, any vector
20 capable of being maintained, of self-replicating or of propagating in a host cell in order to induce the expression of a polynucleotide or a polypeptide may be used.

The techniques for constructing these vectors
25 and the techniques for inserting a suitable sequence into these vectors are widely described in the literature (see in particular Sambrook et al., Molecular Cloning: A Laboratory Manual, 1989).

Advantageously, the vectors according to the invention comprise at least one origin of replication. Preferably, the vectors of the invention also comprise at least one selection marker and preferably a

5 selection marker which can be used in plant cells or in plants. Among the selection markers, mention may be made of the genes for resistance to antibiotics, such as the *nptII* gene for canamycin resistance (Bevan et al., Nature 304:184-187, 1983) and the *hph* gene for

10 hygromycin resistance (Gritz et al., Gene 25:179-188, 1983). Mention will also be made of the genes for tolerance to herbicides, such as the *bar* gene (White et al., NAR 18:1062, 1990) for bialaphos tolerance, the EPSPS gene (US 5,188,642) for glyphosate tolerance or

15 the HPPD gene (WO 96/38567) for isoxazole tolerance. Use may also be made of the genes encoding easily identifiable reporter enzymes, such as the GUS enzyme, or genes encoding pigments and enzymes which regulate the production of pigments, in the transformed cells.

20 Such selection marker genes are in particular described in patent applications EP 242 236, EP 242 246, GB 2 197 653, WO 91/02071, WO 95/06128, WO 96/38567 or WO 97/04103.

Advantageously, these vectors are used for

25 transforming a host organism. Those skilled in the art will choose the suitable transformation vectors in particular as a function of the host organism to be

transformed and as a function of the transformation technique used.

For transforming plant cells or plants, it will in particular be a virus which can be used for the transformation of developed plants, and which also contains its own elements for replication and for expression. Preferentially the vector for transforming the plant cells or plants according to the invention is a plasmid.

Many vectors have been developed for transforming plants with *Agrobacterium tumefaciens*. Other vectors are used for the transformation techniques which are not based on the use of *Agrobacterium*. These vectors are well known to those skilled in the art and widely described in the literature.

Transformation

A subject of the invention is also a process for transforming host organisms, in particular plant cells, with an *SGS3* polynucleotide, an expression cassette, a transformation vector or an expression vector according to the invention.

According to the present invention, the transformation of the host organism may be obtained by any suitable known means; the transformation techniques, and in particular the techniques for transforming plants, are fully described in the

specialized literature. For the processes for transforming plant cells and for regenerating plants, mention will be made in particular of the following patents and patent applications: US 4,459,355,
 5 US 4,536,475, US 5,464,763, US 5,177,010, US 5,187,073, EP 267 159, EP 604 662, EP 672 752, US 4,945,050, US 5,036,006, US 5,100, 792, US 5,371,014, US 5,478,744, US 5,179,022, US 5,565,346, US 5,484,956, US 5,508,468, US 5,538,877, US 5,554,798, US 5,489,520,
 10 US 5,510,318, US 5,204,253, US 5,405,765, EP 442 174, EP 486 233, EP 486 234, EP 539 563, EP 674 725, WO 91/02071, WO 95/06128 and WO 99/19497.

Some techniques use *Agrobacterium* in particular for transforming dicotyledons. A series of
 15 methods consist in using, as a means for transfer into the plant, a chimeric gene inserted into an *Agrobacterium tumefaciens* Ti plasmid or *Agrobacterium rhizogenes* Ri plasmid. Other methods consist in bombarding cells, protoplasts or tissues with particles
 20 to which the DNA sequences are attached. Other methods may also be used, such as microinjection or electroporation, or direct precipitation using PEG.

Those skilled in the art will choose the suitable method depending on the nature of the host
 25 organism, in particular of the plant cell or of the plant.

Host organisms

The present invention also relates to a host organism transformed with an SGS3 polynucleotide, an expression cassette or a vector according to the invention.

According to the invention, the term "host organism" is intended to mean in particular any monocellular or multicellular, lower or higher organism, in particular chosen from bacteria, yeasts, fungi or plant cells and plants. Advantageously, the bacteria are chosen from *Escherichia coli*, the yeasts are chosen from *Pichia pastoris* and *Saccharomyces cerevisiae*, and the fungi are chosen from *Aspergillus niger*. Preferentially, the host organism is a plant cell or a plant.

According to the invention, the term "plant cell" is intended to mean any cell derived from a plant and which may constitute undifferentiated tissues such as calluses, differentiated tissues such as embryos, parts of plants, plants or seeds.

According to the invention, the term "plant" is intended to mean any differentiated multicellular organism capable of photosynthesis, in particular monocotyledons or dicotyledons, more particularly crop plants, which may or may not be intended for animal or human food, such as maize, wheat, barley, sorghum, rapeseed, soybean, rice, sugar cane, beetroot, tobacco, cotton, clover, duckweed (*lemnae*), etc.

According to a particular embodiment of the invention, the host organism comprises at least one other heterologous gene encoding a peptide, a polypeptide or a protein of interest. The

5 polynucleotide comprising an *SGS3* polynucleotide according to the invention and the other heterologous gene(s) may have been introduced into the host organism simultaneously by means of the same vector comprising them or by means of several vectors, or sequentially by

10 means of several vectors, or alternatively by crossing several host organisms, each comprising a heterologous gene.

According to the invention, the term "heterologous gene" is intended to mean any gene

15 introduced artificially into the host organism, and more particularly integrated artificially into its genome, the methods allowing this introduction or integration possibly being those described previously, the content of the references cited being incorporated

20 herein by way of reference.

The heterologous gene, other than the *SGS3* polynucleotides according to the invention, may be a gene comprising a coding sequence and the 5' and 3' regulatory elements for said coding sequence, which are

25 not modified compared to the natural gene, reintroduced artificially into the genome of a host organism which may be of the same species as that from which the gene was isolated, or a different species. The heterologous

gene may also be a chimeric gene or an expression cassette comprising a coding sequence of plant, bacterial, fungal, viral or animal origin, under the control of regulatory elements which are functional in
5 the host organism and which are different from those naturally functionally linked to the coding sequence.

A subject of the present invention is also the plants containing transformed cells as defined above, in particular the plants regenerated from the
10 transformed cells and their descendants. The regeneration is obtained using any suitable process, which depends on the nature of the species, as described for example in the references above.

The present invention also relates to the
15 genetically modified plants into the genome of which an SGS3 polynucleotide or an expression cassette according to the invention are integrated in a manner which is stable and transmissible by sexual reproduction.

The present invention also relates to plants
20 obtained by crossing the regenerated plants above with other plants. It also relates to the seeds of transformed plants.

sgs3 mutants

The invention also relates to the *sgs3* mutants in which the *SGS3* gene is inactivated. The inactivation of this gene leads to inhibition of the post-transcriptional inactivation phenomena in these mutants.

The inactivation of the *SGS3* gene in the plants may be obtained by means of various mutagenesis, site-directed mutagenesis or "gene machine" techniques, or using homologous recombination techniques (Kempin, S.A. et al., Targeted disruption in *Arabidopsis*, Nature 389:802-803, 1997). These techniques are well known to those skilled in the art. Among the mutagenesis techniques, mention will be made of the chemical mutagenesis techniques. Mention will also be made of the mutagenesis techniques using transposable elements which allow inactivation of genes by insertion. When the mutagenesis techniques used do not make it possible to specifically inactivate the *SGS3* gene, the mutants obtained are screened in order to identify the mutants affected in the *SGS3* gene. This screening may be phenotypic screening or screening based on the amplification and sequencing of the *SGS3* gene in the mutants, according to techniques described in the literature. Among the site-directed mutagenesis techniques, mention will be made of chimeraplasty (US 6,010,907).

In one particular embodiment of the invention, mutants are obtained according to the process described by Elmayan et al. (Plant Cell, 10:1747-1757, 1998) by treating seeds with a solution
5 of EMS (ethyl methanesulfonate) at 0.4%. The mutants are then analyzed in order to identify the mutants affected in the *SGS3* gene. This screening may, for example, be carried out by PCR.

The present invention also relates to the use
10 of *SGS3* mutants for identifying *SGS3* genes in other plant species, such as for example tobacco, rapeseed, sunflower, soybean, cotton, rice, maize, sorghum, barley or wheat. The functional homologs of *SGS3* in other species are identified by complementation of the
15 *sgs3* mutants according to the invention. A polynucleotide which restores the wild-type phenotype of post-transcriptional inactivation is cloned. The sequence of this polynucleotide is then determined in order to identify the constituent elements of the
20 cloned gene.

Inhibition/inactivation of *SGS3* and expression of heterologous genes in plants

The development of genetic transfer
25 techniques has allowed the expression of genes in plants, in particular with a view to improving their agronomic properties or for the production of proteins of interest. However, post-transcriptional inactivation

phenomena constitute a considerable obstacle to the stability of transgene expression in plants. These phenomena of suppression of the expression of the transgene are particularly frequent in the context of
5 strongly expressed transgenes. The present invention relates to a novel plant *SGS3* gene. The inhibition or inactivation of this *SGS3* gene in plants causes inhibition of the post-transcriptional inactivation phenomenon and therefore makes it possible to produce
10 plants in which the expression of heterologous genes is more stable, and also plants in which the level of expression of the heterologous genes is higher.

Inactivation/inhibition of the *SGS3* gene in plants

In a first embodiment, the invention relates
15 to a process for expressing a heterologous gene in a plant, characterized in that it comprises transforming the plant with the heterologous gene and inhibiting the expression of the *SGS3* gene in said plant.

Preferably, the invention relates to a
20 process for expressing a heterologous gene in a plant, characterized in that it comprises the following steps:
a) said plant is transformed with said heterologous gene; and
b) the expression of an *SGS3* polynucleotide according
25 to the invention is inhibited in said plant.

Preferentially, the inhibition of the expression of the *SGS3* gene comprises transforming the

a) an antisense polynucleotide of the coding sequence of an *SGS3* plant gene;

- c) an expression cassette comprising, in the direction of transcription, a promoter which is functional in a host organism, a polynucleotide as defined in a) or b) and a terminator sequence which is functional in said host organism.

15 plant with the heterologous gene and inactivating the
expression of the *SGS3* gene in said plant.

A subject of the invention is also a process for expressing a heterologous gene in a plant, comprising the following steps:

- 20 a) said plant is transformed with said heterologous gene;
- b) the expression of an SGS3 polynucleotide according to the invention is inactivated in said plant.

In the context of the present invention, it is clearly understood that the step for inactivating or inhibiting the plant *SGS3* gene and the step for transforming the plant with a heterologous gene may be carried out simultaneously on the same plant or

sequentially, or alternatively by crossing several plants. The processes according to the invention may therefore also comprise steps for regenerating plants, for asexual multiplication or for crossing plants.

5 Heterologous genes

Various heterologous genes of interest may be expressed in the plants in which the expression of the SGS3 gene is inhibited or inactivated. Preferably, the heterologous gene encodes peptides, proteins or
10 enzymes. They may be reporter proteins, selection markers, or peptides or proteins of interest which confer novel properties on the host organism, more particularly novel agronomic properties for the transformed plants.

15 Among the genes which confer novel agronomic properties on the transformed plants, mention may be made of the genes which confer tolerance to certain herbicides, those which confer resistance to certain insects, those which confer tolerance to certain
20 diseases, etc. Such genes are in particular described in patent applications WO 91/02071 and WO 95/06128.

Among the genes which confer tolerance to certain herbicides, mention may be made of the Bar gene which confers bialaphos tolerance, the gene encoding a
25 suitable EPSPS which confers resistance to herbicides having EPSPS as the target, such as glyphosate and its salts (US 4,535,060, US 4,769,061, US 5,094,945, US 4,940,835, US 5,188,642 US 4,971,908, US 5,145,783,

US 5,310,667, US 5,312,910, US 5,627,061, US 5,633,435,
FR 2 736 926), the gene encoding glyphosate
oxydoreductase (US 5,463,175), or a gene encoding an
HPPD which confers tolerance to herbicides having HPPD
5 as the target, such as isoxazoles, in particular
isoxafutole (FR 95 06800, FR 95 13570), diketoneitriles
(EP 496 630, EP 496 631) or triketones, in particular
sulcotrione (EP 625 505, EP 625 508, US 5,506,195).
Such genes encoding an HPPD conferring tolerance to
10 herbicides having HPPD as the target are described in
patent application WO 96/38567.

Among the proteins of interest which confer
novel properties of resistance to insects, mention will
be made more particularly of the Bt proteins widely
15 described in the literature and well known to those
skilled in the art. Mention will also be made of the
proteins extracted from bacteria such as Photorabdus
(WO 97/17432 & WO 98/08932).

Among these proteins or peptides of interest
20 which confer novel properties of resistance to
diseases, mention will be made in particular of
chitinases, glucanases and oxalate oxidase, all these
proteins and their coding sequences being widely
described in the literature, or antibacterial and/or
25 antifungal peptides, in particular peptides of less
than 100 amino acids rich in cysteines, such as plant
defensins or thionine, and more particularly lytic
peptides of many origins comprising one or more

disulfide bridges between the cysteines and regions comprising basic amino acids, in particular the following lytic peptides: androctonin (WO 97/30082 and PCT/FR98/01814, filed on August 18, 1998) or drosomycin
5 (PCT/FR98/01462, filed on July 8, 1998).

According to a particular embodiment of the invention, the protein or peptide of interest is chosen from fungal elicitor peptides, in particular elicittins (Kamoun et al., 1993; Panabières et al., 1995).

10 Mention may also be made of the genes which modify the constitution of the modified plants, in particular the content and the quality of certain essential fatty acids (EP 666 918) or the content and the quality of the proteins, in particular in the
15 leaves and/or the seeds of said plants. Mention will be made in particular of the genes encoding proteins enriched in sulfur-containing amino acids (Korit et al., Eur. J. Biochem. 195:329-334, 1991; WO 98/20133; WO 97/41239; WO 95/31554; WO 94/20828; WO 92/14822).

20 These proteins enriched in sulfur-containing amino acids will also have the function of trapping and storing excess cysteine and/or methionine, making it possible to avoid the possible problems of toxicity linked to overproduction of these sulfur-containing
25 amino acids by trapping them. Mention may also be made of genes encoding peptides rich in sulfur-containing amino acids and more particularly in cysteines, said peptides also having antibacterial and/or antifungal

activity. Mention will be made more particularly of plant defensins, and also lytic peptides of any origin, and more particularly the following lytic peptides: androctonin (WO 97/30082 and PCT/FR98/01814, filed on 5 August 18, 1998) or drosomycin (PCT/FR98/01462, filed on July 8, 1998).

The host organisms of the present invention may also be used for producing proteins of interest in plants or "molecular farming". Specifically, the 10 invention also relates to transformed plants which make it possible to produce higher levels of expression of heterologous genes. Among the proteins of interest, mention will be made in particular of mammalian peptides and proteins. The production of 15 immunoglobulins (US 5,990,385; US 5,639,947, 5,959,177) and of interferon (US 4,956,282) have, for example, been described in plants.

All the methods or operations described below in the examples are given by way of example and 20 correspond to a choice made from the various methods available to achieve the same result. This choice has no bearing on the quality of the result and, consequently, any suitable method may be used by those skilled in the art to achieve the same result. Most of 25 the methods for engineering DNA fragments are described in "Current Protocols in Molecular Biology" Volumes 1 and 2, Ausubel F.M. et al. or in Sambrook et al. 1989.

Description of the figuresFIGURE 1: *sgs3* Mutants

5

ExamplesExample 1Isolation and identification of the *SGS3* gene of
Arabidopsis

10 The *SGS3* mutant (affected in the *SGS3* plant
gene) was obtained using the same experimental protocol
as that which allowed the isolation of the *sgs1* and
sgs2 mutants (Elmayan et al., Plant Cell 10:1747-1757,
1998). The starting line was the L1 line. L1 is a
15 transgenic line obtained by transforming plants of the
Columbia ecotype with the 23b construct (Elmayan and
Vaucheret, Plant J. 9:787-797, 1996). The L1 line
comprises only a single transgenic locus. The
glucuronidase activity in the L1 line is 4000 nmol of
20 4-methylumbelliferone per minute and per microgram of
total proteins in the first days of development. This
activity then decreases very rapidly to become less
than 5 nmol of 4-methylumbelliferone per minute and per
microgram of total proteins 11 days after germination.
25 The inactivation of the expression of the 35S-uidA
transgene is post-transcriptional, as demonstrated in
the run-on experiments revealing strong transcription
of the 35S-uidA transgene in the L1 plants showing very

low GUS activity (Elmayan et al., Plant Cell 10:1747-1757, 1998). For producing mutant plants of the L1 line, 3000 seeds of the L1 line were soaked for 16 hours in a solution of EMS (ethyl methanesulfonate) at 5 0.4%. The seeds were then sown and the plants produced were cultivated under glass until self-fertilization seeds were produced. These seeds were again sown under glass and, in the plants produced, the GUS activity was measured 1 month after germination. The plants 10 exhibiting high activity at this stage were crossed with plants of the Columbia ecotype (to verify that the transgenic locus remains sensitive to post-transcriptional inactivation), backcrossed with the L1 line (to evaluate the state of recessiveness vs 15 dominance of the mutations produced) and crossed with one another (to classify the various mutants produced into complementation groups, each group defining a gene). 6 independent *sgs3* mutants were thus isolated. These 6 mutations are recessive. The GUS activity in 20 these 6 mutant lines, one month after germination, is between 2500 and 3500 nmol of 4-methylumbelliferone per minute and per microgram of total proteins. The GUS activity in these mutant lines, 1 month after germination, is between 2500 and 3500 nmol of 4- 25 methylumbelliferone per minute and per microgram of total proteins. In order to confirm that the *sgs3* mutations affect the expression of the 35S-*GUS* transgene at the transcriptional level, the GUS

activity, the mRNA accumulation and the transcription rate were measured by fluorimetric tests, by mRNA blot analysis and by "run-on" experiments. The GUS activity is multiplied by a factor of 300 in the *sgs2* mutants compared to the L1 line, while the mRNA accumulation is multiplied by a factor of 250. The transcription rate is only multiplied by a factor of 2.6 compared to the L1 line. In order to verify that the *sgs33* mutations protected against the post-transcriptional inactivation of a gene other than the *uidA* gene, one of the *sgs3* mutants (named *sgs3-2*) was crossed with the 2a3 line (Elmayan et al., Plant Cell 10:1747-1757, 1998). The 2a3 line is a transgenic *Arabidopsis thaliana* line which results from the transformation of a plant of the Columbia ecotype with the 2a construct (Elmayan et al., Plant Cell 10:1747-1757, 1998) containing the transcribed portion of the *Arabidopsis NIA2* gene encoding nitrate reductase under the control of the 35S promoter and the hygromycin resistance gene *hpt*. All the plants of the 2a3 line which are homozygous for the 2a construct exhibit post-transcriptional inactivation of the *Nia2* genes (transgenic and endogenous), leading to chlorosis of the plant and then to its death. When the transgenic 2a3 locus is in the heterozygous state, only some of the plants undergo the post-transcriptional inactivation. The stage at which this inactivation occurs is variable from one plant to the other. In some plants, the inactivation is sufficiently

late to allow the production of pollen and of seeds. The hybrid plants derived from the cross between the *sgs3-2* mutant and the 2a3 line were cultivated under glass and the self-fertilization seeds were harvested.

5 The seeds were sown under glass and the plants produced which exhibited no chlorosis were kept in order to harvest their self-fertilization seeds. We then sowed the various batches of seeds on an agar medium containing 20 mg/l of hygromycin. Among these, some

10 gave only plants which were resistant to hygromycin and showed no sign of chlorosis throughout their development. Among these lines of resistance to the post-transcriptional inactivation of the nitrate reductase genes, some were also homozygous for the 23b

15 construct. We also showed that the plants of all these lines exhibited high GUS activity throughout their development. These results therefore show that the *sgs3* mutation not only protects against the post-transcriptional inactivation of the 35S-*uidA* transgene,

20 but also of the *NIA2* endogenous genes and transgenes. Some of these lines resistant to the post-transcriptional inactivation of the nitrate reductase genes and homozygous for the 2a3 locus no longer contained the 23b construct. These plants were named

25 SGS3-2 2a3.

In order to determine the biological role of the gene corresponding to the *sgs3* mutations, *sgs3-1* mutants were inoculated with the cucumber mosaic virus.

(CMV) strain I17F. On the wild-type plants, infection with this viral strain produces plants in which development is slower and modified: smaller leaves of the rosette, long but very flexible floral scape, fertility decreased but not zero. In the *sgs3-1* mutants, infection with this viral strain produces an increased modification of development: the plants have a particularly bushy habit, the leaves of the rosette are small and tendrilled, the floral scape reaches, at the end of development, a size of about 5 cm, and the plants are completely sterile. These experiments therefore show that the gene corresponding to the *sgs3* mutations makes it possible to limit the negative effects on development caused by CMV virus infection.

Two mutants, *sgs3-1* and *sgs3-2*, were crossed with plants of the Landsberg ecotype. From these hybrid (F1) plants resulting from these crosses, the self-fertilization seeds were harvested. These seeds were sown in vitro on an agar medium containing 50 mg/l of canamycin in order to select the plants (F2) containing the 23b transgene. These canamycin-resistant plants were planted out and cultivated under glass. The GUS activity in these plants was measured at various stages of their development. Only the plants exhibiting high GUS activity throughout development (and therefore homozygous for the *sgs3* mutation) were kept and the self-fertilization seeds were harvested. 120 F2 lines homozygous for the *sgs3-1* mutation (F2-1 lines) and 90

F2 lines homozygous for the *sgs3-2* mutation (F2-2 lines) were thus produced. The self-fertilization seeds from each of these lines were sown under glass and, for each line, a pool of plants was harvested in order to
 5 extract the DNA therefrom. These DNAs were used to locate the *sgs3* mutations on the *Arabidopsis* genome. The initial locating was carried out using the F2-1 lines. The F2-2 lines then allowed us to verify that the *sgs3-2* mutation was located in the same region of
 10 the genome as the *sgs3-1* mutation. These analyses show that the *sgs3* mutations were located between the 13H2L and 3B3D molecular markers. The polymorphism corresponding to the 13H2L molecular marker was revealed by hybridization (of the Southern blot type)
 15 of the total DNA of *Arabidopsis* plants, digested with the HindIII restriction enzyme, with a radioactive DNA fragment corresponding to the left end of the 13H2 yeast artificial chromosome (YAC) (13H2L probe). The polymorphism corresponding to the 3B3D molecular marker
 20 was revealed by hybridization (of the Southern blot type) of the total DNA of *Arabidopsis* plants, digested with the HindIII restriction enzyme, with a radioactive DNA fragment corresponding to the right end of the 3B3 YAC (3B3 probe).

25 We were able to determine, by molecular hybridization of the Southern type, on a membrane onto which the DNA of a bacteria artificial chromosome library (BAC IGF) was transferred, with the radioactive

DNA fragment corresponding to the 13H2L and 3B3D probes, that these 2 DNA fragments hybridized on the same BAC: BAC F20I20. These results therefore show that the *sgs3-1* and *sgs3-2* mutations affect a DNA sequence
5 included in the BAC F20I20.

DNA from the BAC F20I20 was then purified. It was partially digested with the *Sau3AI* restriction enzyme. The resulting DNA fragments were cloned at the *BamHI* site of the transfer DNA of the binary plasmid
10 (allowing the transformation of plants via *Agrobacterium*) pBin+. The resulting plasmids were introduced into *E. coli* and then into the *Agrobacterium tumefaciens* strain C58pMP90. The resulting bacterial strains were used to transform plants of the *sgs3-2* 2a3
15 lines. The bacterial strain 356 made it possible to produce 20 transgenic lines. Among these 20 lines, 19 showed signs of chlorosis identical to those observed on the 2a3 line. On 3 of these plants, we were able to show, by hybridization of the northern type using the
20 *Arabidopsis thaliana* NIA2 gene as the probe, that this chlorosis resulted from the non-accumulation of the transcripts of the nitrate reductase genes (transgenic and endogenous) and was therefore due to the post-transcriptional inactivation of the nitrate reductase
25 genes. Among these 19 plants, 2 gave self-fertilization seeds. The plants derived from these seeds, cultivated under glass, also showed signs of chlorosis.

The DNA sequence which was inserted at the BamHI site of the pBin+ plasmid and which had led to the isolation of the bacterial strain 356 was determined. Subclones of the 356 clone were produced in
5 the pBin+ vector and the same *sgs3-2 2a3* line was transformed with these subclones in order to determine those capable of restoring the function of the *SGS3* gene. The smallest subclone capable of restoring this function constitutes the *SGS3* gene such as it is
10 described in this patent. It was possible to predict the ORF of *SGS3* by computer analysis. The sequence of the cDNA containing the ORF of the *SGS3* gene, and therefore the position of the promoter, terminator and intronic sequences of *SGS3*, were verified after having
15 isolated and cloned this sequence. In order to isolate, we first performed a reverse-transcription reaction using *Arabidopsis thaliana* total RNA. We then performed a PCR reaction on this pool of cDNA using the pair of primers p356AD' (AAAATGAGTTCTAGGGCTGGTCC) and P356Y'
20 (GTCTCAATCATCTTCATTGTGAAGGCC). These primers are located at the 2 ends of the ORF of *SGS3*. This PCR product was cloned and sequenced.

Using the BLAST program, no significant homology could be found between the *SGS3* sequence
25 (nucleotide or amino acid) and any sequence present in the data bases.

Example 2Analysis of the *sgs3* mutants

The sequence of the *SGS3* gene was determined in 5 *sgs3* mutants. A PCR reaction was carried out on the genomic DNA of these 5 mutants using the p356AD' and P356Y' primers (see Example 1). This reaction made it possible to amplify the entire *SGS3* gene. The fragment amplified by this PCR reaction was sequenced.

Five distinct point mutations were thus identified in the various *sgs3* mutants. These mutations correspond to four stop codons and one amino acid change. The various mutations observed in the *sgs3* mutants are represented in Figure 1. The amino acid marked in bold indicates the position of the mutation in the *SGS3* polypeptide. * indicates the presence of a stop codon and () indicates a new amino acid substituted for the amino acid marked in bold affected by the mutation.

Example 3Construction of expression cassettes for the overexpression and inhibition of *SGS3*

A PCR-type reaction is first carried out on *Arabidopsis thaliana* complementary DNA using, as primers, the following oligonucleotides:

p356AD' : AAAATGAGTTCTAGGGCTGGTCC

P356Y' : GTCTCAATCATCTTCATTGTGAAGGCC

The nucleotide sequence thus obtained is then treated with the "klenow" enzyme in order to generate "blunt" ends at the ends of the amplified sequence. The sequence is then cloned between the 35S promoter and the terminator of the cauliflower mosaic virus, at the SmaI site of the pRT100 vector.

For the overexpression of *SGS3*, the clones are selected such that the sequence corresponding to p356AD' is located close to the 35S promoter. For the inhibition of *SGS3*, the clones are selected such that the sequence corresponding to p356Y' is located close to the 35S promoter. This *Assgs3* construct allows the expression of an antisense mRNA for the *SGS3* mRNA.

15

Example 4

Transformation of plants

The expression cassettes constructed as described above are then introduced into a binary vector so as to allow their introduction, via *Agrobacterium tumefaciens* into plants. The binary vector used is the pBIN+ plasmid (Van Engelen et al., Transgenic Research 4, 288-290, 1995). This is performed by digesting the constructs obtained above, with the SphI enzyme (which releases the expression cassettes), and ligating the product of this digestion to the pBIN+ plasmid digested with the SphI enzyme.

Example 5Inhibition of expression of the SGS3 plant gene with
antisense sequences

The complete cDNA of the *SGS3* plant gene was
5 cloned, in the antisense orientation (aSGS3), between
the 35S promoter (p35S) and the 35S terminator (t35S).
The chimeric gene p35S-aSGS3-t35S was re-cloned into
the pBiB-Hyg binary vector and then transferred into
Agrobacterium tumefaciens. Plants of the L1 line (p35S-
10 GUS-tRbcS gene subjected to PTGS) were transformed by
soaking in agrobacteria. The transformed plants were
selected on medium supplemented with hygromycin. The
GUS activity of the p35S-GUS-tRbcS transgene was
measured in the nontransformed L1 plants, in 28
15 hygromycin-resistant transformants and also in the *sgs3*
mutants obtained by EMS mutagenesis of the L1 line. The
GUS activity in the nontransformed L1 plants is between
0 and 10 nmol MU/min/ μ g of proteins, while the GUS
activity in the *sgs3* mutants is between 3000 and
20 5500 nmol MU/min/ μ g of proteins. 11 of the 28
hygromycin-resistant transformants showed a GUS
activity of between 3000 and 5500 nmol MU/min/ μ g of
proteins, showing that the *SGS3* plant gene may be
inhibited by the chimeric gene p35S-aSGS3-t35S, thus
25 mimicking an *sgs3* mutation.

Claims

1. A polynucleotide, characterized in that
it comprises a polynucleotide chosen from the following
5 polynucleotides:

- a) the polynucleotide of SEQ ID No. 1, and
- b) the polynucleotide of SEQ ID No. 2.

2. A polynucleotide, characterized in that
it comprises a polynucleotide chosen from the following
10 polynucleotides:

- a) a polynucleotide capable of selectively
hybridizing to a polynucleotide as claimed in
claim 1, and
- b) a polynucleotide at least 80% homologous to a
15 polynucleotide as claimed in claim 1.

3. The polynucleotide as claimed in claim
2, characterized in that it restores an sgs3 mutant of
Arabidopsis thaliana.

4. A polynucleotide, characterized in that
20 it comprises the polynucleotide the sequence of which
is between position 1 and position 695 of SEQ ID No. 1.

5. A polynucleotide, characterized in that
it comprises a polynucleotide chosen from the following
polynucleotides:

- 25 a) a polynucleotide capable of selectively
hybridizing to a polynucleotide as claimed in
claim 4, and

- b) a polynucleotide at least 80% homologous to a polynucleotide as claimed in claim 4.

6. The polynucleotide as claimed in claim 5, characterized in that it has promoter activity in plant cells and plants.

7. A polypeptide, characterized in that it comprises the polypeptide of SEQ ID No. 3.

8. A polypeptide, characterized in that it comprises a polypeptide chosen from the following polypeptides:

- a) a biologically active fragment of the polypeptide of SEQ ID No. 3; and
b) a polypeptide at least 80% homologous to the polypeptide of SEQ ID No. 3.

9. The polypeptide as claimed in claim 8, characterized in that it restores an sgs3 mutant of *Arabidopsis thaliana*.

10. A polynucleotide, characterized in that it comprises a polynucleotide encoding a polypeptide as claimed in one of claims 7-9.

11. An expression cassette, characterized in that it comprises, in the direction of transcription:

- a) a promoter which is functional in a host organism; and
b) a polynucleotide as claimed in one of claims 1-3 and 10; and
c) a sequence which is a terminator in said host organism.

12. An expression cassette, characterized in that it comprises, in the direction of transcription:

- d) a promoter which is functional in a host organism; and
- 5 e) a polynucleotide as claimed in one of claims 1-3 and 10, in the antisense orientation; and
- f) a sequence which is a terminator in said host organism.

13. An expression cassette, characterized in that it comprises, in the direction of transcription:

- a) a polynucleotide as claimed in one of claims 4-6;
- b) a polynucleotide encoding a heterologous polypeptide;
- c) a sequence which is a terminator in plant cells or
- 15 plants.

14. An expression vector or transformation vector, comprising a polynucleotide as claimed in one of claims 1-6 and 10 or an expression cassette as claimed in one of claims 11-13.

20 15. A process for transforming host organisms, in particular plant cells or plants, by integrating into said host organism at least one polynucleotide as claimed in one of claims 1-6 and 10 and/or at least one expression cassette as claimed in

25 one of claims 11-13 and/or at least one vector as claimed in claim 14.

16. A process for expressing a heterologous gene in a plant, characterized in that it comprises the following steps:

- a) said plant is transformed with said heterologous gene; and
- b) the expression of a polynucleotide as claimed in one of claims 1-3 and 10 is inhibited in said plant.

17. The process as claimed in claim 16, characterized in that step b) comprises transforming said plant with an expression cassette as claimed in claim 12.

18. A process for expressing a heterologous gene in a plant, characterized in that it comprises the following steps:

- b) said plant is transformed with said heterologous gene;
- c) the expression of a polynucleotide as claimed in one of claims 1-3 and 10 is inactivated in said plant.

19. A transformed host organism comprising at least one polynucleotide as claimed in one of claims 1-6 and 10 and/or of at least one expression cassette as claimed in one of claims 11-13 and/or of at least one vector as claimed in claim 14.

20. The host organism as claimed in claim 19, characterized in that it comprises at least one

heterologous gene encoding a peptide or a protein of interest.

21. The host organism as claimed in either of claims 19 and 20, characterized in that the host
5 organism is chosen from bacteria, yeasts, fungi, plant cells or plants.

22. The host organism as claimed in claim 21, characterized in that the plants are chosen from maize, wheat, barley, sorghum, rapeseed, soybean, rice,
10 beetroot, tobacco and cotton.

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(54) Title: NOVEL SGS3 PLANT GENE AND USE THEREOF

(54) Titre: NOUVEAU GENE SGS3 DE PLANTE ET SON UTILISATION

(57) Abstract: The invention concerns novel polynucleotides comprising the SGS3 plant gene involved in post-transcriptional inactivation phenomena in transgenic plants and in the resistance of plants to viral infections, and its use for preparing genetically modified plants.

(57) Abrégé: La présente invention concerne de nouveaux polynucléotides comprenant le gène SGS3 de plante impliqué dans les phénomènes d'inactivation post-transcriptionnelle dans les plantes transgéniques et dans la résistance des plantes aux infections virales, et son utilisation pour la préparation de plantes génétiquement modifiées.

WO 01/05951 A2

**COMBINED DECLARATION
AND POWER OF ATTORNEY**

(Original, Design, National Stage of PCT, Divisional, Continuation or C-I-P Application)

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name; I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

NOVEL SGS3 PLANT GENE AND USES THEREOF

This declaration is of the following type:

- ☐ original
- ☐ design
- ☒ national stage of PCT/FR00/02052
- ☐ divisional
- ☐ continuation
- ☐ continuation-in-part (C-I-P)

the specification of which: (complete (a), (b), or (c))

- (a) ☐ is attached hereto.
- (b) ☒ was filed on January 11, 2002 as Application Serial No. 10/030,829 and was amended on (if applicable).
- (c) ☐ was described and claimed in PCT International Application No. filed on and was amended on (if applicable).

Acknowledgement of Review of Papers and Duty of Candor

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the patentability of the subject matter claimed in this application in accordance with Title 37, Code of Federal Regulations § 1.56.

☐ In compliance with this duty there is attached an information disclosure statement. 37 CFR 1.98.

Priority Claim

I hereby claim foreign priority benefits under Title 35, United States Code, § 119(a)-(d) of any foreign application(s) for patent or inventor's certificate or of any PCT International Application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT International Application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application on which priority is claimed

(complete (d) or (e))

- (d) ☐ no such applications have been filed.
- (e) ☒ such applications have been filed as follows:

PRIOR FOREIGN/PCT APPLICATION(S) FILED WITHIN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO SAID APPLICATION				
COUNTRY	APPLICATION NO.	DATE OF FILING (day, month, year)	DATE OF ISSUE (day, month, year)	PRIORITY CLAIMED UNDER 35 USC 119
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Claim for Benefit of Prior U.S. Provisional Application(s)

I hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below:

Provisional Application Number	Filing Date

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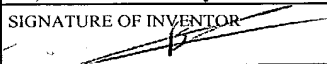
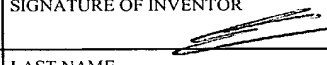

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DATE <u>11/03/2002</u>	SIGNATURE OF INVENTOR 			
FULL NAME OF FOURTH JOINT INVENTOR, IF ANY	LAST NAME	FIRST NAME	MIDDLE NAME	
RESIDENCE & CITIZENSHIP	CITY	STATE or FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP	
POST OFFICE ADDRESS	POST OFFICE ADDRESS	CITY	STATE or COUNTRY	ZIP CODE
DATE	SIGNATURE OF INVENTOR			
FULL NAME OF FIFTH JOINT INVENTOR, IF ANY	LAST NAME	FIRST NAME	MIDDLE NAME	
RESIDENCE & CITIZENSHIP	CITY	STATE or FOREIGN COUNTRY	COUNTRY OF CITIZENSHIP	
POST OFFICE ADDRESS	POST OFFICE ADDRESS	CITY	STATE or COUNTRY	ZIP CODE
DATE	SIGNATURE OF INVENTOR			

BAKER BOTTS, L.L.P.

FILE NO.: A34920-PCT-USA (072667.0179)

FULL NAME OF SIXTH JOINT INVENTOR, IF ANY	LAST NAME	FIRST NAME	MIDDLE NAME	
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FULL NAME OF SEVENTH JOINT INVENTOR, IF ANY	LAST NAME	FIRST NAME	MIDDLE NAME	
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DATE	SIGNATURE OF INVENTOR			

Check proper box(es) for any added page(s) forming a part of this declaration

- ☐ Signature for ninth and subsequent joint inventors. Number of pages added : ____.
- ☐ Signature by administrator(trix), executor(trix) or legal representative for deceased or incapacitated inventor.
Number of pages added ____.
- ☐ Signature for inventor who refuses to sign, or cannot be reached, by person authorized under 37 CFR 1.47.
Number of pages added ____.

10 Nov 1999 10/030829
8 APR 2002

<110> Beclin, Christophe
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Vaucheret, Herve

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atg	gtt	gta	aag	gag	ctg	agg	cag	atc	tct	gag	gac	aat	cag	cag	ctg	1344	
Met	Val	Val	Lys	Glu	Leu	Arg	Gln	Ile	Ser	Glu	Asp	Asn	Gln	Gln	Leu		
		435					440					445					
aac	tac	ttt	aag	aac	aag	ctc	tca	aaa	cag	aac	aag	cac	gcc	aag	gtg	1392	
Asn	Tyr	Phe	Lys	Asn	Lys	Leu	Ser	Lys	Gln	Asn	Lys	His	Ala	Lys	Val		
	450					455					460						
ctt	gag	gaa	tct	ctg	gaa	att	atg	agc	gag	aag	ctg	cgt	aga	act	gca	1440	
Leu	Glu	Glu	Ser	Leu	Glu	Ile	Met	Ser	Glu	Lys	Leu	Arg	Arg	Thr	Ala		
465					470					475					480		
gag	gat	aat	cgg	atc	gtg	aga	cag	aga	act	aag	atg	cag	cat	gaa	cag	1488	
Glu	Asp	Asn	Arg	Ile	Val	Arg	Gln	Arg	Thr	Lys	Met	Gln	His	Glu	Gln		
				485					490					495			
aac	agg	gaa	gag	atg	gat	gca	cac	gac	agg	ttt	ttc	atg	gat	tca	atc	1536	
Asn	Arg	Glu	Glu	Met	Asp	Ala	His	Asp	Arg	Phe	Phe	Met	Asp	Ser	Ile		
		500						505					510				
aaa	cag	atc	cat	gaa	aga	aga	gac	gca	aag	gag	gag	aat	ttc	gag	atg	1584	
Lys	Gln	Ile	His	Glu	Arg	Arg	Asp	Ala	Lys	Glu	Glu	Asn	Phe	Glu	Met		
		515					520					525					
ttg	cag	cag	cag	gaa	cgt	gcc	aag	gtt	gtt	ggc	cag	cag	cag	cag	aac	1632	
Leu	Gln	Gln	Gln	Glu	Arg	Ala	Lys	Val	Val	Gly	Gln	Gln	Gln	Gln	Asn		
	530					535					540						
att	aat	ccc	tct	agc	aat	gac	gat	tgc	cga	aag	aga	gct	gag	gaa	gtg	1680	
Ile	Asn	Pro	Ser	Ser	Asn	Asp	Asp	Cys	Arg	Lys	Arg	Ala	Glu	Glu	Val		
545					550					555					560		
tca	agc	ttc	atc	gag	ttt	caa	gag	aaa	gag	atg	gag	gag	ttt	gtg	gaa	1728	
Ser	Ser	Phe	Ile	Glu	Phe	Gln	Glu	Lys	Glu	Met	Glu	Glu	Phe	Val	Glu		
				565					570					575			
gag	agg	gag	atg	ctg	ata	aaa	gat	caa	gag	aag	aag	atg	gaa	gac	atg	1776	
Glu	Arg	Glu	Met	Leu	Ile	Lys	Asp	Gln	Glu	Lys	Lys	Met	Glu	Asp	Met		
			580					585						590			

<400>	3													
Met-Ser	Ser Arg Ala Gly Pro Met Ser Lys Glu Lys Asn Val Gln Gly													
1	5						10					15		
Gly Tyr Arg Pro Glu Val Glu Gln Leu Val Gln Gly Leu Ala Gly Thr														
	20						25					30		
Arg Leu Ala Ser Ser Gln Asp Asp Gly Gly Glu Trp Glu Val Ile Ser														
	35					40					45			
Lys Lys Asn Lys Asn Lys Pro Gly Asn Thr Ser Gly Lys Thr Trp Val														
50					55					60				
Ser Gln Asn Ser Asn Pro Pro Arg Ala Trp Gly Gly Gln Gln Gln Gly														
65				70					75					80
Arg Gly Ser Asn Val Ser Gly Arg Gly Asn Asn Val Ser Gly Arg Gly								90					95	
	85													
Asn Gly Asn Gly Arg Gly Ile Gln Ala Asn Ile Ser Gly Arg Gly Arg							105					110		
	100													
Ala Leu Ser Arg Lys Tyr Asp Asn Asn Phe Val Ala Pro Pro Pro Val														
	115					120					125			
Ser Arg Pro Pro Leu Glu Gly Gly Trp Asn Trp Gln Ala Arg Gly Gly														
130					135					140				
Ser Ala Gln His Thr Ala Val Gln Glu Phe Pro Asp Val Glu Asp Asp														
145				150					155					160
Val Asp Asn Ala Ser Glu Glu Glu Asn Asp Ser Asp Ala Leu Asp Asp														
	165							170					175	
Ser Asp Asp Asp Leu Ala Ser Asp Asp Tyr Asp Ser Asp Val Ser Gln														
	180						185					190		
Lys Ser His Gly Ser Arg Lys Gln Asn Lys Trp Phe Lys Lys Phe Phe														
195						200				205				
Gly Ser Leu Asp Ser Leu Ser Ile Glu Gln Ile Asn Glu Pro Gln Arg														
210					215					220				
Gln Trp His Cys Pro Ala Cys Gln Asn Gly Pro Gly Ala Ile Asp Trp														
225				230					235					240
Tyr Asn Leu His Pro Leu Leu Ala His Ala Arg Thr Lys Gly Ala Arg														
	245							250					255	
Arg Val Lys Leu His Arg Glu Leu Ala Glu Val Leu Glu Lys Asp Leu														
	260						265					270		
Gln Met Arg Gly Ala Ser Val Ile Pro Cys Gly Glu Ile Tyr Gly Gln														
275					280					285				
Trp Lys Gly Leu Gly Glu Asp Glu Lys Asp Tyr Glu Ile Val Trp Pro														
290					295					300				
Pro Met Val Ile Ile Met Asn Thr Arg Leu Asp Lys Asp Asp Asn Asp														
305				310					315					320
Lys Trp Leu Gly Met Gly Asn Gln Glu Leu Leu Glu Tyr Phe Asp Lys														
	325							330					335	
Tyr Glu Ala Leu Arg Ala Arg His Ser Tyr Gly Pro Gln Gly His Arg														
	340						345					350		

[illegible]